IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: 1611	**Customer No.: 035811**
Examiner	: Gina C. Yu	
Serial No.	: 10/785,237	
Filed	: February 24, 2004	
Inventors	: Yves Millou	Docket No.: 1026-04
	: Katia Fontes	
	: Cécile Tourel	Confirmation No.: 1050
Title	: COSMETIC COMPOSITION COMPRISING	
	: AN ESSENTIAL OIL EXTRACTED FROM	
	: HELICHRYSUM ITALICUM	
		Dated: July 22, 2009

RESPONSE

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is in response to the Official Action mailed January 22, 2009.

Claims 1-3, 7-8, 10, 14-16 and 19-24 are pending. Claims 1-3, 7-8, 10, 14-16 and 19-24 are rejected.

Claims 1-3, 8, 14-16, 20 and 22-24 are rejected as obvious under 35 USC §103(a) over the combination of Amrita and Spina.

Claims 7, 10, 19 and 21 are rejected as obvious under 35 USC §103(a) over the combination of Amrita, Spina and US '954.

Claims 1-3, 8, 14-16, 20 and 22-24 are not obvious under 35 USC §103(a) over the combination of Amrita and Spina.

Claims 7, 10, 19 and 21 are not obvious under 35 USC §103(a) over the combination of Amrita, Spina and US '954.

First, Amrita discloses the use of *Helichrysum italicum* for tissue regeneration and for skin-rejuvenation. The *Helichrysum italicum* flowers alone are used. However, Amrita is silent not only about the amounts of essential oil used for producing the composition, but also about the amounts of neryl acetate comprised in the essential oil. Both of these features are critical

features of the claimed compositions and contribute to the unexpected results obtained, and shown, in the previously submitted Exhibits.

Second, Spina does not teach these features or otherwise correct this deficiency of Amrita. Spina discloses a composition for hair restoration. This composition comprises several different essential oils -- only one of which is *Helichrysum italicum* essential oil. The amount of *Helichrysum italicum* essential oil is from 3.67 to 4.33 wt% whereas in the claimed compositions, it is from 0.1 to 2.1 wt%. Spina is also silent about the amounts of neryl acetate present in the *Helichrysum italicum* essential oil. Furthermore, Spina does not have the same goal as the claimed compositions or as the compositions of Amrita. This means that one of ordinary skill in the art would not be motivated to combine Spina or Amrita on studying the teachings of these documents. Additionally, Spina discloses compositions containing different amounts of *Helichrysum italicum* essential oil than the claimed compositions. This leads to the conclusion that, even if one of ordinary skill in the art was to combine Spina with Amrita, this combination would not result in the claimed compositions. Thus, because Amrita and Spina are silent about the amount of neryl acetate, it is clear that this element of the claimed compositions, by itself or in combination with the other elements of the claims, would not have been obvious to a person of ordinary skill in the art.

Third, US '954 fails to correct these deficiencies of the core combination of Amrita and Spina.

Fourth, the Applicants submit evidence of nonobviousness by way of Exhibits 1, 2 and 3 (enclosed) which shows the claimed compositions produce unexpected results. The results of Exhibit 1 demonstrate in an art accepted model for protein glycation induced aging that the claimed compositions, as tested, efficiently prevent protein glycation such as that associated with aging. The results in Exhibit 2 demonstrate in an art accepted human epidermis model the claimed compositions, as tested, have significant anti-free radical formation activity and inhibited lipoperoxidation as assessed using a malonaldehyde based assay to measure lipoperoxidation. The results in Exhibit 3 demonstrate in the claimed compositions, as tested, stimulate collagen III synthesis and maturation in human dermal fibroblasts. All the results obtained are entirely unexpected relative to the knowledge in the art and the disclosures of Amrita, Spina and US '954 cited in the rejections. Thus, the compositions of Claims 1-3, 8, 14-16, 20 and 22-24 are not obvious over the combination of Amrita and Spina, and the

compositions of Claims 7, 10, 19 and 21 are not obvious over the combination of Amrita, Spina and US '954 because these compositions produce unexpected results relative to the disclosures of these references.

Altogether, this means the claims are not obvious over the various combinations of Amrita, Spina and US '954 relied on in the rejections.

The Applicants respectfully request the withdrawal of the rejections under 35 USC §103(a).

In light of the foregoing, the Applicants respectfully submit that the entire application is now in condition for allowance, which is respectfully requested.

Respectfully submitted,

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EXHIBIT 1

NIGHT CARE CREAM *IMMORTELLE*
Preventing glycation of proteins

Principle :

Glycation is the reaction that results in a covalent bonding between a target protein and a sugar, in particular glucose.
In an organism it is a slow spontaneous reaction, i.e. non-enzymatic, that takes place in the extra-cellular environment.

This phenomenon corresponds to an alteration of the target protein. Glycation increases with aging. It partially explains collagene reticulation.

An *in vitro* model was selected to assess the product's effects. It relies on measuring the formation of derivate products of the albumine and glucose-6-phosphate glycation reaction. Some of these derivates (AGE) are fluorescent. The reaction has been measured after a 15 days incubation.

Studied product :

NIGHT CARE *IMMORTELLE*

In parallel, a reference molecule has been tested : aminoguanidine

Procedure :

Trial system (TS) :
The trial system is reaction mix containing bovine albumine (0,5 g/ml) and glucose (500 mM) in phosphate buffer (0,2 M, pH=7,4).

Products incubation+TS :
Products are mixed with the trial system in sterile tubes ; the tubes are covered with aluminum foil (the reaction must take place sheltered from light)

Concerning experimental conditions where some of the reagents or products are missing, the volume of the tube must be completed with MilliQ water.

After sealing the tubes with parafilm (the reaction must take place sheltered from oxygen), the different reaction mixes are placed in a drying oven 8 days at 37°C.

Effects evaluation :

After 8 days incubation, 100µl of each tube is taken and transferred in a black 96 well plate.

Then fluorescence is read with FLUOstar (*BMG*) (excitation : 355 nm ; emission : 460 nm)

Results are expressed in **arbitrary fluorescence units**.

« product+ bovine albumine » (= interference) sample related fluorescence is subtracted from « product+bovine albumine+ glucose » data.

Results :

After 8 days incubation of bovine albumine with a glucose overload, glycation « cross-linking » formation is observed. It has been assessed using fluorescence proprieties of the bovine albumine-glucose adduct.

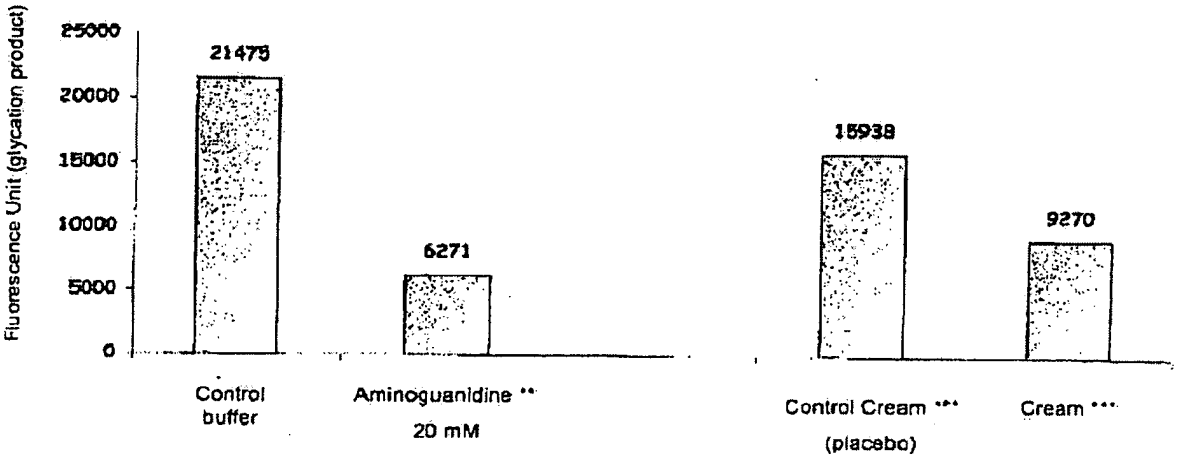
In experimental conditions, **NIGHT CARE *IMMORTELLE*** present in the trial system prevents cross-linking in between bovine albumine and glucose quite efficiently (see figure on next page).

OCCITANE

« IMMORTELLE »

CREAM

Effect on the bovine albumine and glucose* glycation reaction



* Fluorescence measure of the glycated protein ** Positive control in buffer

*** 5% product in buffer - fluorescence measure of the under-natent

EXHIBIT 2

NIGHT CARE CREAM "IMMORTELLE"
Anti radical properties

1. DISPLAY OF THE ANTI FREE RADICAL PROTECTION ACCREDITED TO NIGHT CARE *IMMORTELLE'S* ACTION :

This property has been shown by MDA dosage which is a lipoperoxydation index.

In order to show this property, we used Skin Ethic® (Nice, France), an *in vitro* reconstituted epidermis model which allows the dosage of different biochemical markers.

BIBLIOGRAPHIC REFERENCES :

CULTURE PROCEDURE :

Human origin keratinocytes are inoculated on 0,63 cm^2 or 4 cm^2 polycarbonate filters in a defined media (modified MCDB 153) and supplemented.

Cells are cultured during 14 days at the air/liquid interface. Culture media is changed every two days.

Thus formed epidermis has been used for the study starting the 17th day of culture.

STUDIED PRODUCT :

NIGHT CARE *IMMORTELLE* has been applied on the surface of each epidermis, at the rate of 2 mg/cm^2.

2. DOSAGE METHODS

Epidermis have been treated (24h), washed (iced NaCl 0,9%) and collected.
The tissues have been homogenised in a Tris HCl buffer volume (1ml), 20 mM, pH 7,4 at 4°C with a glass polygrinder.
Each homogenate is taken and centrifuged at 300g and 4°C during 10mn.
Supernatant is taken and used for our dosage (500 µl).
50 µl BHT (2% in absolute ethanol) are added in each supernatant.

2.1 TBARS (THIOBARBITURIC ACID-REACTIVE SUBSTANCES) DOSAGE :

- The complexation reaction is initiated by 1 ml of TBA 0,375% (in 0,25M HCl containing 15% Trichloracetic acid).
- 1 hour incubation in a 80°C water bath.
- The reaction is stopped by a brutal cooling in ice (20 minutes).
- Extraction of the molecules that reacted with the TBAR is done by adding 300 µl butan-1-ol strongly shaked with a vortex.
- A 10 min. 3000 rpm centrifugation is necessary to get the organic phase.
- Each butanolic phase is collected and transferred in the wells of a microtitration black plate.
- Reading by spectrofluorometry (*FLUOstar, BMG*) at 515 nm excitation wavelength, the reading being done at 553 nm.

- The samples are compared to a standard 1,1,3,3- tetrametoxypropan (*Malonaldehyde bis [dimathyl acetal], Sigma #T-1642*) scale, to determine MDA concentration in the solution.

2.2 PROTEIN DOSAGE :

For each sample we determine a protein concentration of the collected tissue's content.
The procedure consists of associating to this content (50 µl) a mix (100µl) of a bicinchoninic acid (BCA) solution and of a Copper sulfate solution. Cu++ ions of this mixture are reduced to Cu+ in presence of proteins. These ions chellate with 2 BCA molecules.
Samples are read with a spectrophotometer (*Dynatech reader*) at 550 nm and compared to a Bovine Serum Albumine (BSA) standard scale to determine the total protein concentration (in BSA equivalents) of the solution.

3. CALCULATION OF THE MDA CONCENTRATION :

Final results are expressed in : **MDA nM / protein mg** .

4. RESULTS (GRAPHIC) :

In the selected experimental conditions , the **NIGHT CARE *IMMORTELLE*** product applied on reconstituted epiderms in a defined media, had a significant anti free radical activity.

Indeed, we could notice an inhibitory action on lipoperoxydation **(~ 50% protection)**.

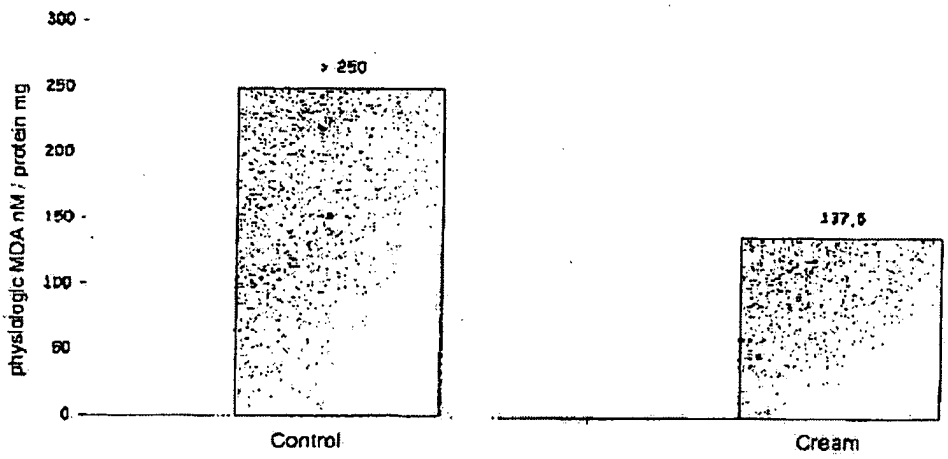
OCCITANE

« IMMORTELLE »

CREAM

Anti-radicalair activity

Physiological lipoperoxydation measure by MDA* dosage



* Fluorescence measure of malonaldehyde (MDA)
Dosage after 24 hours application on reconstituted epiderm

EXHIBIT 3

BIOalternatives

Study report: GT080450
Proposal: GT080450

Effect of the compound HE immortelle Bio Corse on collagen III production by fibroblasts

STUDY PROMOTER

L'OCCITANE

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The investigators and the author of this report hereby certify the validity of the data presented and attest their full agreement with the conclusions presented at the end of the report.

June 25, 2008

Study director: Franck JUCHAUX, MS
Cellular biology Manager

TABLE OF CONTENT

1. INTRODUCTION

An effect of the compound **HE immortelle Bio Corse** on the collagen III production by dermal fibroblasts was researched.

In this present study, the activity of this compound was evaluated on the stimulation of collagen III synthesis/maturation using an immunofluorescent labelling on human dermal fibroblasts.

ABBREVIATIONS

AU	Arbitrary unit
BSA	Bovine serum albumin
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
FCS	Foetal calf serum
MTT	3-(4,5-dimethyl thiazol-2-yl)-2,5 diphenyl-tetrazolium bromide)
NHDF	Normal human dermal fibroblast
OD	Optical density
PBS	Phosphate buffered saline
PFA	Paraformaldehyde
RT	Room temperature
Sd	Standard deviation
sem	Standard error of the mean
TGF	Transforming growth factor

2. MATERIALS AND METHODS

2.1. Biological model

- Cellular type: Normal human dermal fibroblasts (NHDF) pool PF2 used at the 9th passage
- Culture conditions: 37°C, 5% CO_2
- Culture medium: DMEM (Invitrogen 21969035) supplemented with
  Glutamine 2 mM (Invitrogen 25030024)
  Penicillin 50 UI/ml - Streptomycin 50 µg/ml (Invitrogen 15070063)
  Foetal calf serum (FCS) 10% (Invitrogen 10270098)

2.2. Test compound and reference

Test compound	Aspect	Stock solution	Dilution	Test concentrations
HE Immortelle Bio Corse Batch n° OC0611367 Ref. MPBi00iM01 GT080116-3	• Liquid • Storage at RT	1% in DMSO	Culture medium	$8x10^{-5}$, $4x10^{-4}$ and $2x10^{-3}$%

Reference	Stock solution	Dilution	Test concentration
TGF-ß (R&D Systems 240-B-010)	2 µg/ml	Culture medium	10 ng/ml

2.3. Cytotoxicity preliminary assay

- plate format: 96-well
- cells/well: 4000 NHDF in DMEM 10% FCS
- replicates: 3
- concentration range: see Table 1
- cells/compound contact: 72 hours
- evaluation parameter: MTT reduction assay and morphological observations with microscope (objective x10)

2.4. Culture and treatment

The fibroblasts were cultivated in 96-well plates in culture medium. At subconfluence, the medium was removed and replaced by culture medium containing or not (control) the test compound or the reference. The cells were then incubating for 72 hours. All conditions were performed in n=3.

2.5. Collagen III production by normal human fibroblasts - immunofluorescence

After incubation, culture media were eliminated. The cells were rinsed with phosphate buffered saline (PBS) solution and fixed with paraformaldehyde (PFA) 4% solution. After saturation of non specific antigenic sites by incubation in PBS-Tween, bovine serum albumin (BSA) 5% buffer, cells were labelled with a primary antibody anti-collagen III (TEBU 600-401-1051) for 1 hour at room temperature.

The primary antibody was then revealed using a fluorescent secondary antibody (GAR-Alexa 488) and the cell nuclei were coloured with Hoechst solution (bis-benzimide).

The acquisition of the images was performed with the INCell Analyzer™ 1000 (GE Healthcare). Controls without primary antibody were performed in order to adjust the acquisition parameters of the camera. Five photos were taken per well. The labelling was quantified by the measurement of the fluorescence intensity (Integration of numerical data with the Developer Toolbox 1.5, GE Healthcare software).

2.6. Data management

The raw data was analysed with Microsoft Excel®.

The inter-group comparisons were performed by Student's t-test. The statistical analysis can be interpreted if $n \geq 5$, however for $n < 5$ the statistical values are for information only.

Formula used in this report:

Standard error of the mean: $sem = Sd/\sqrt{n}$

The standard error of the mean (sem) is a measure of how far the sample mean is likely to be from the true population mean. The sem is calculated as the sd divided by the square root of sample size.

Percentage of Stimulation:

$$\text{Stimulation (\%)} = \left[\frac{\text{Value}}{\text{Mean of control}} \times 100 \right] - 100$$

Percentage of viability: $\%\ \text{viability} = (OD_{\text{sample}} / OD_{\text{control}}) \times 100$

3. RESULTS

3.1. Cytotoxicity preliminary assay

Table 1

The results of the MTT reduction assay and the observation of the cell layers determined, in accordance with the study promoter, the concentrations to be tested (see paragraph 2.2).

3.2. Collagen III production by on normal human fibroblasts

Table 2 and Figure 1

The reference TGF-β significantly stimulated collagen III production by fibroblasts. This result was expected and validated the assay.

The compound **HE immortelle Bio Corse** tested at $8x10^{-5}$% and $4x10^{-4}$% significantly stimulated collagen III production by fibroblasts. At the highest concentration, no effect was observed.

4. CONCLUSION

To conclude, the compound **HE immortelle Bio Corse** show a stimulating effect on collagen III synthesis/maturation by human dermal fibroblasts.

5. TABLES AND FIGURE

Table 1: Effect of the compound **HE Immortelle Bio Corse** on the viability of fibroblasts

	Control		HE Immortelle Bio Corse stock solution prepared at 1% in DMSO							Unit %
			1.3E-07	6.4E-07	3.2E-06	1.6E-05	8.0E-05	4.0E-04	0.002	0.01
Viability (%)	98	101	95	96	102	103	100	97	97	48
	97	107	93	91	98	104	101	103	102	54
	100	97	99	95	98	105	101	107	105	53
Mean	100		96	94	99	104	100	102	101	52
sem	1		2	1	1	0	0	3	2	2
morphological Observations	+		+	+	+	+	+	+	+	-

Legend
+: normal population ; +/-: growth reduction ; -: toxicity ; 0 : cells mortality
g: grains of compound ; op: opacity of the compound ; *: morphological modification ; ag: agglutinated cells
sem : Standard error of the mean (standard deviation divided by sample size square root)

Table 2: Effect of the compound **HE immortelle Bio Corse** on collagen III production by human dermal fibroblasts

Treatment		Basic data					Normalized data		
Treatment	Concentration	Fluorescence Intensity/Number of cells (AU)	Mean (AU)	% Control	sem (%)	$p^{(1)}$	Stimulation (%)	sem (%)	$p^{(1)}$
Control	-	162 156 183	170	100	4	-	0	4	-
TGF-β	10 ng/ml	216 220 210	215	126	2	**	26	2	**
HE Immortelle Bio Corse	$8x10^{-5}$%	192 206 219	206	121	5	*	21	5	*
	$4x10^{-4}$%	187 205 214	202	119	5	*	19	5	*
	$2x10^{-3}$%	195 181 153	176	104	7	ns	4	7	ns

(1): Threshold for statistical significance
ns : > 0.05, Not significant
* : 0.01 to 0.05, Significant
** : 0.001 to 0.01, Very significant
*** : < 0.001, Extremely significant

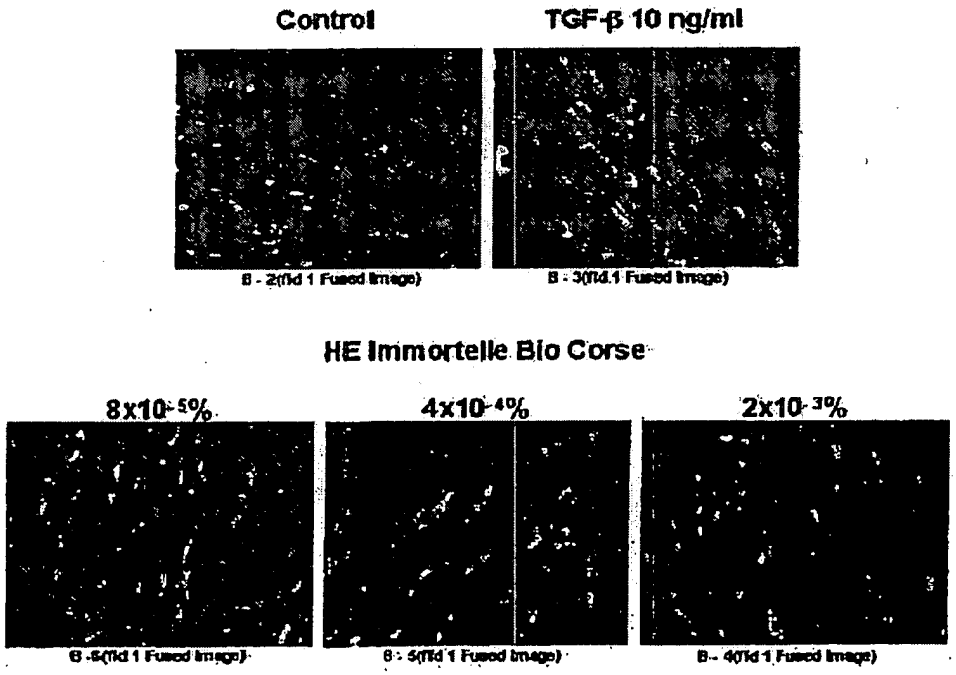

Figure 1: Representative images of collagen III labelling in human fibroblasts